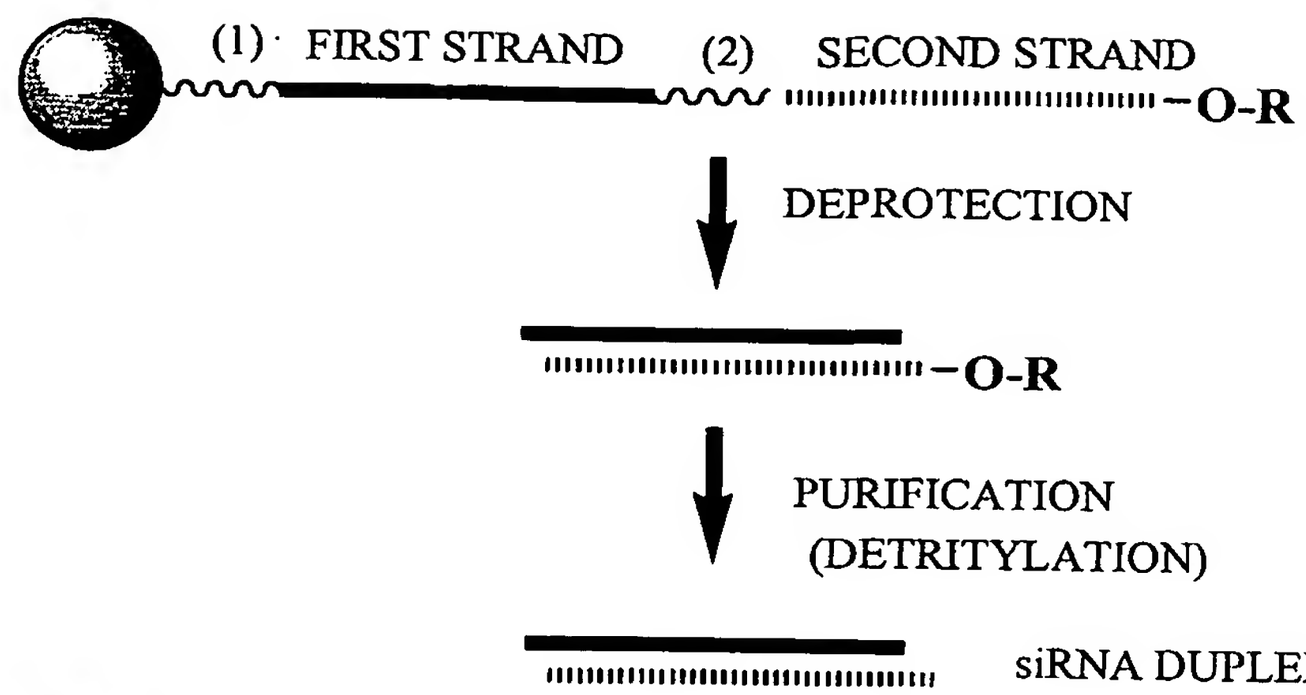
Figure 1

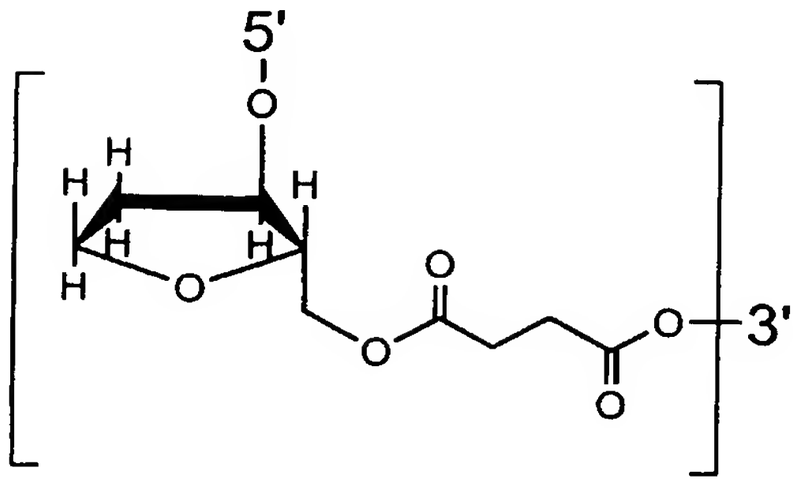


= SOLID SUPPORT

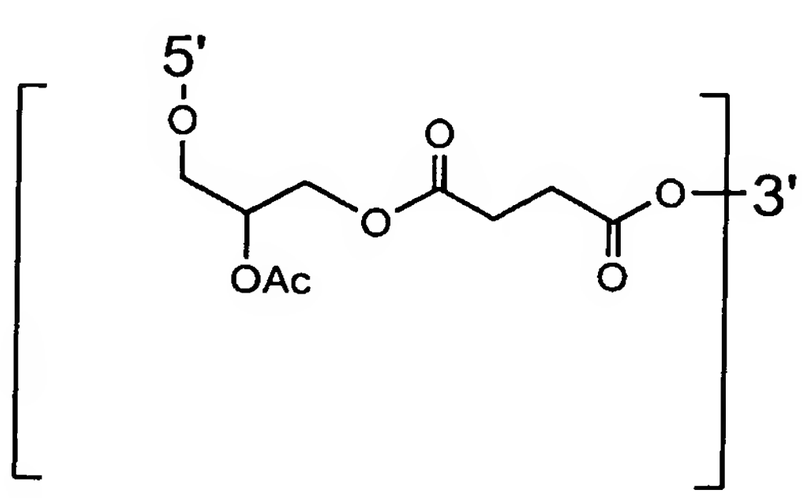
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

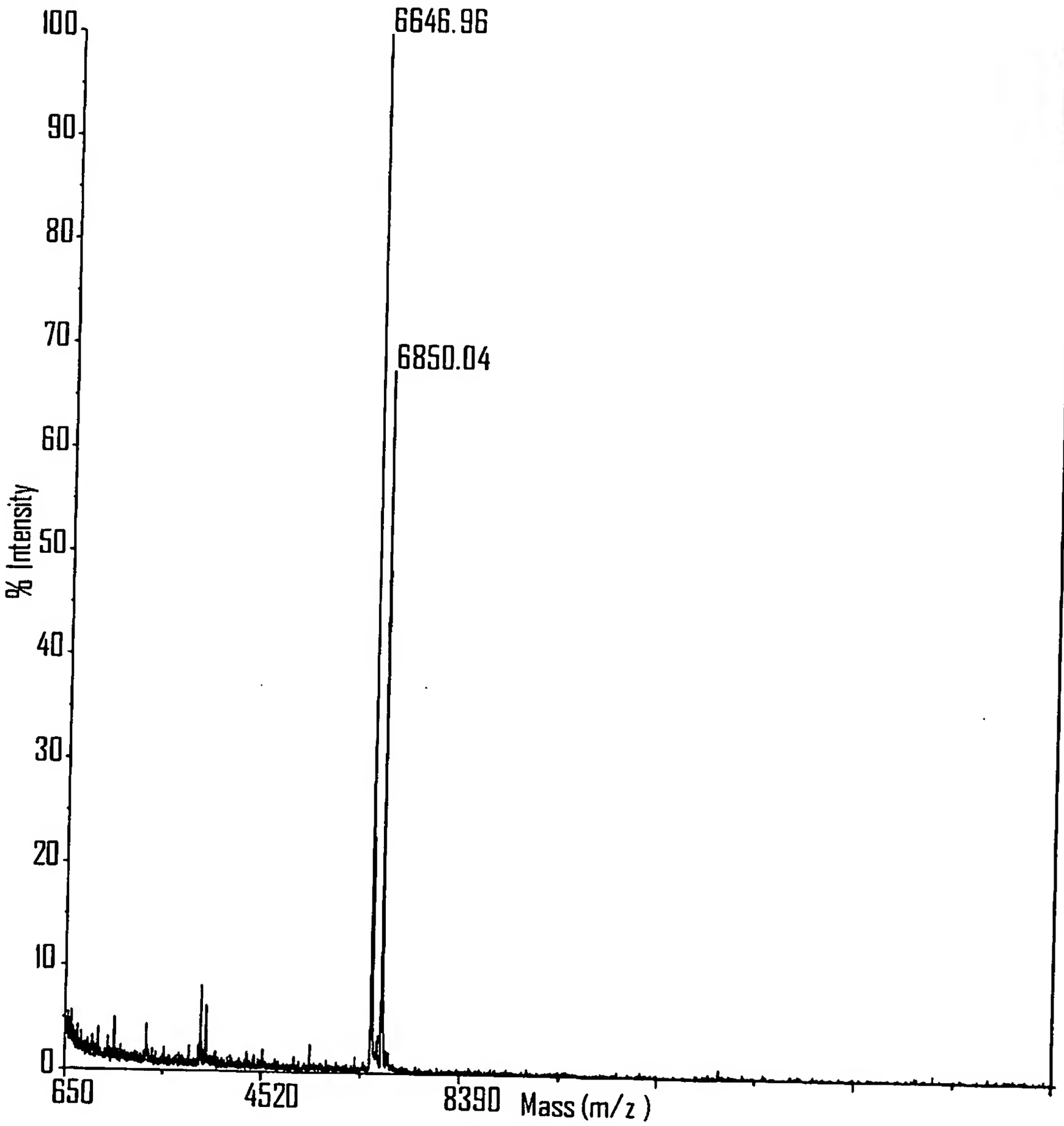


INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2


100
90
80
70
60
50
40
30
20
10
0
% Intensity
6646.96
6850.04
650
4520
8390
Mass (m/z)

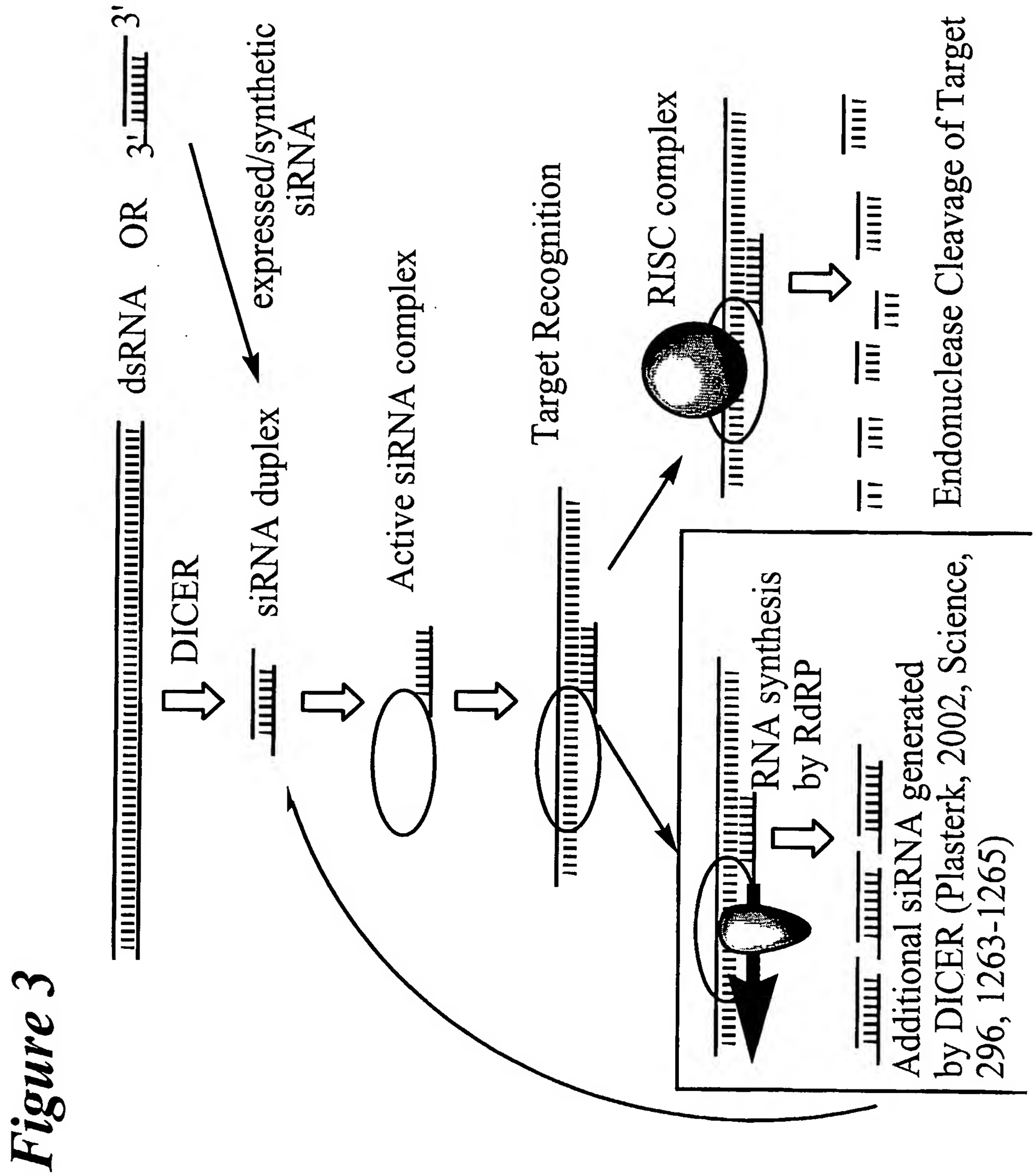

dsRNA OR 3' 3'
expressed/synthetic siRNA
DICER
siRNA duplex
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Endonuclease Cleavage of Target
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)

Figure 3

Figure 4

A

SENSE STRAND (SEQ ID NO 3375)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- ..L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 3376)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

B

SENSE STRAND (SEQ ID NO 3377)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-OM EXCEPT POSITIONS (N N)

5'- N N N N N N N N N N N N N N N N N N N (N_sN) -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 3378)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

C

SENSE STRAND (SEQ ID NO 3379)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 3380)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

D

SENSE STRAND (SEQ ID NO 3381)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 3378)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

E

SENSE STRAND (SEQ ID NO 3382)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N N) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 3378)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

F

SENSE STRAND (SEQ ID NO 3381)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 3383)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENTL = GLYCERYL or B THAT IS OPTIONALLY PRESENTS = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE that is optionally absent

Figure 5

A

SENSE STRAND (SEQ ID NO 3384)

5'- iB-A U G U G C U U G A A G A U C C U U G *T T*-iB -3'

3'- L-T_S*T* U A C A C G A A C U U C U A G G A A C -5'

ANTISENSE STRAND (SEQ ID NO 3385)

B

SENSE STRAND (SEQ ID NO 3386)

5'- a *u g u g c u u* g a a g a *u c c u u g T T* -3'

3'- L-T_S*T u* a c a c g a a *c u u c u* a g g a a *c* -5'

ANTISENSE STRAND (SEQ ID NO 3387)

C

SENSE STRAND (SEQ ID NO 3388)

5'- iB-A u G u G c u u G A A G A u c c u u G *T T*-iB -3'

3'- L-T_S*T u* A *c* A *c* G A A *c u u c u* A G G A A *c* -5'

ANTISENSE STRAND (SEQ ID NO 3389)

D

SENSE STRAND (SEQ ID NO 3390)

5'- iB-*A u G u G c u u G A A G A u c c u u G T T*-iB -3'

3'- L-T_S*T u* a c a c g a a *c u u c u* a g g a a *c* -5'

ANTISENSE STRAND (SEQ ID NO 3387)

E

SENSE STRAND (SEQ ID NO 3391)

5'- iB-A *u* G *u* G *c u u* G A A G A *u c c u u* G *T T*-iB -3'

3'- L-T_S*T u* a c a c g a a *c u u c u* a g g a a *c* -5'

ANTISENSE STRAND (SEQ ID NO 3387)

F

SENSE STRAND (SEQ ID NO 3390)

5'- iB-*A u G u G c u u G A A G A u c c u u G T T*-iB -3'

3'- L-T_S*T u A c A c G A A c u u c u A G G A A c* -5'

ANTISENSE STRAND (SEQ ID NO 3392)

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro

italic lower case = 2'-deoxy-2'-fluoro

underline = 2'-O-methyl

*ITALIC UPPER CASE = DEOXY*iB = INVERTED DEOXYABASICL = GLYCERYL MOIETY or iB
OPTIONALLY PRESENTS = PHOSPHOROTHIOATE (
PHOSPHORODITHIOATE OPTIONALLY PRESEN

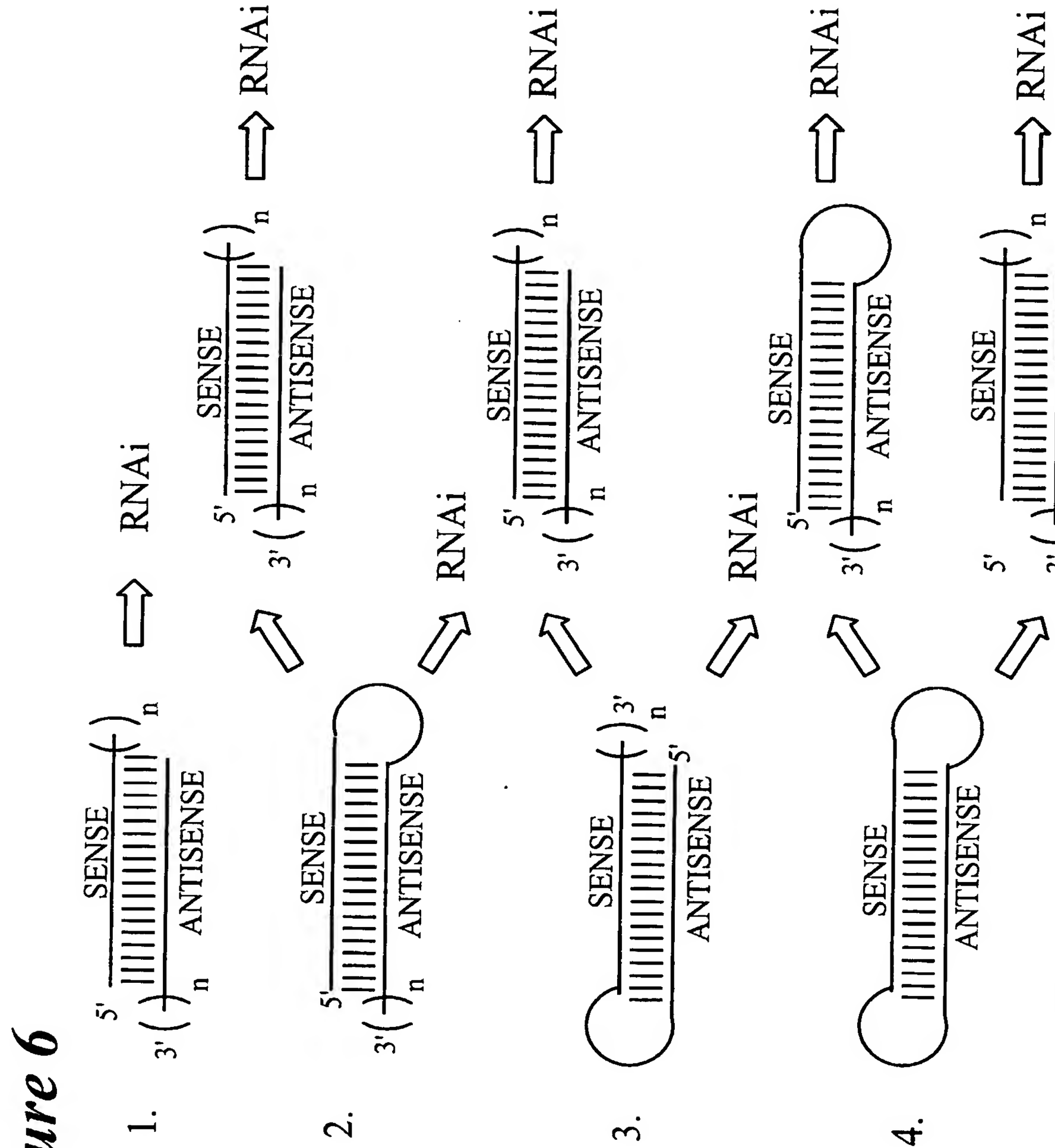


Figure 6

Figure 7

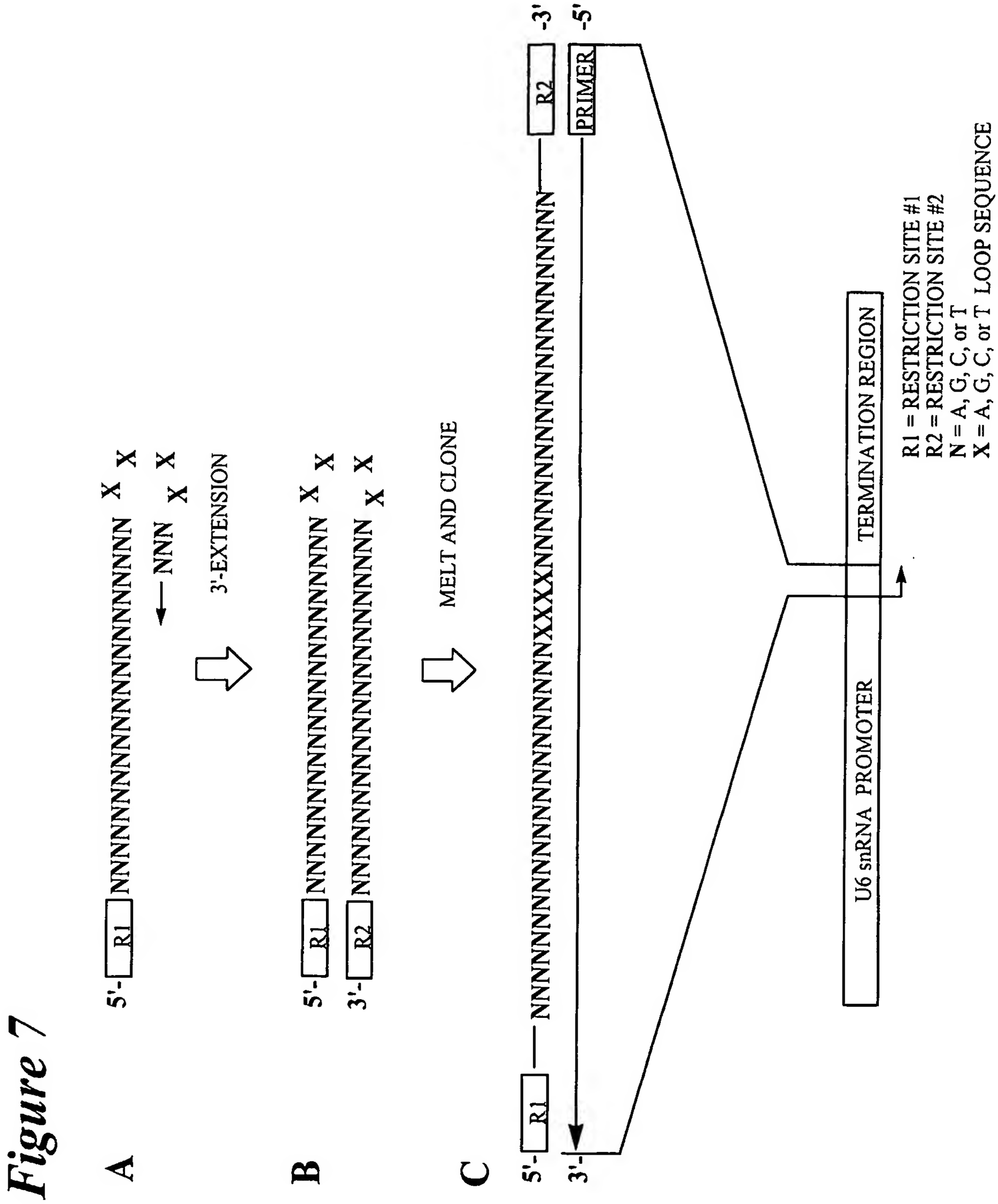
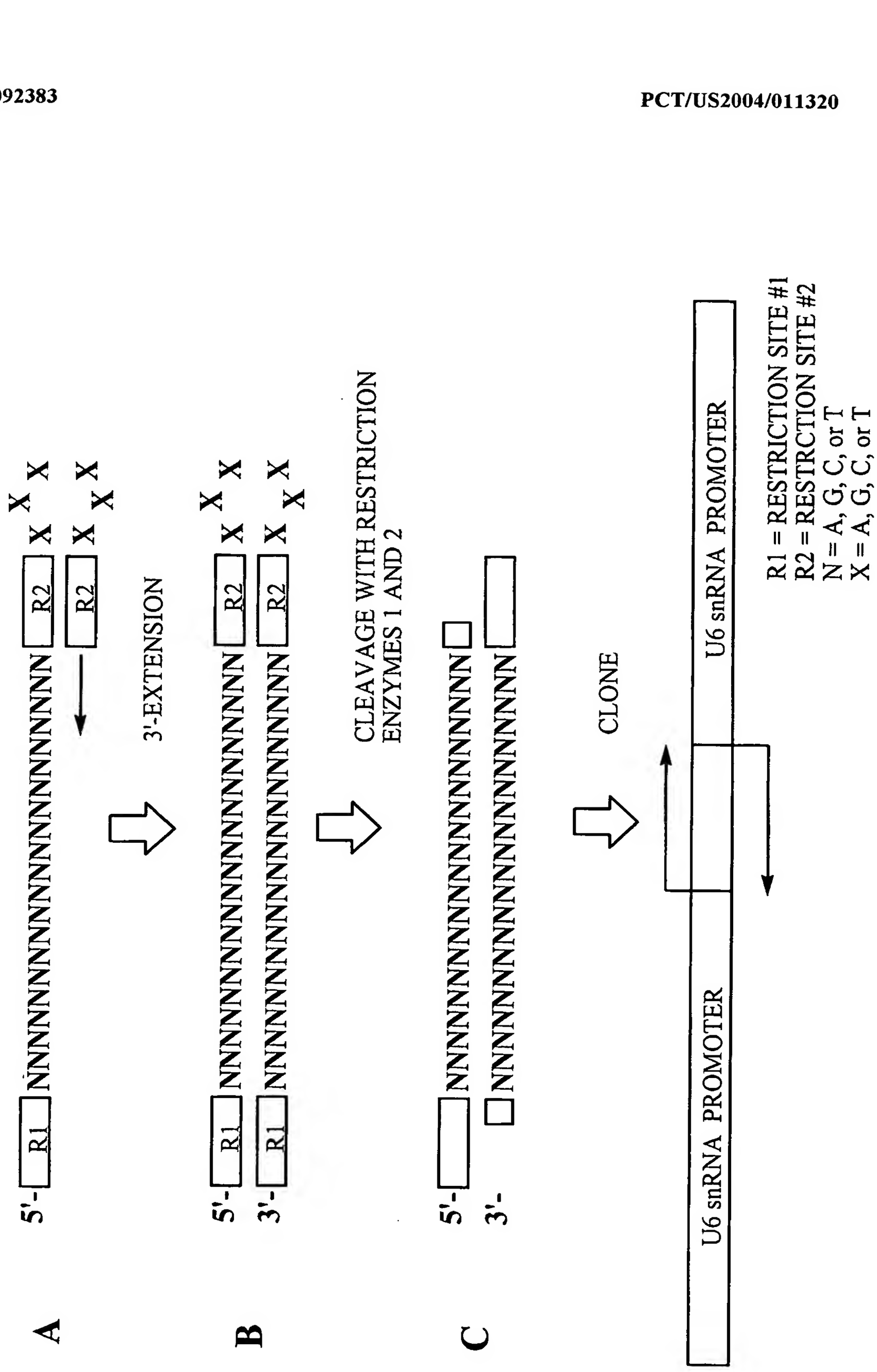

A
5'-
R1
NNNNNNNNNNNNNNNNNNN
X X
NNN
X X
3'-EXTENSION
B
5'-
R1
NNNNNNNNNNNNNNNNNNN
X X
3'-
R2
NNNNNNNNNNNNNNNNNNN
X X
MELT AND CLONE
C
5'-
R1
NNNNNNNNNNNNNNNNXXXXNNNNNNNNNNNNNNNNNNN
3'-
U6 snRNA PROMOTER
TERMINATION REGION
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T LOOP SEQUENCE

Figure 8


A
B
C
5'-
3'-
R1
R2
N
X
3'-EXTENSION
CLEAVAGE WITH RESTRICTION ENZYMES 1 AND 2
CLONE
U6 snRNA PROMOTER
R1 = RESTRICTION SITE #1
R2 = RESTRCTION SITE #2
N = A, G, C, or T
X = A, G, C, or T

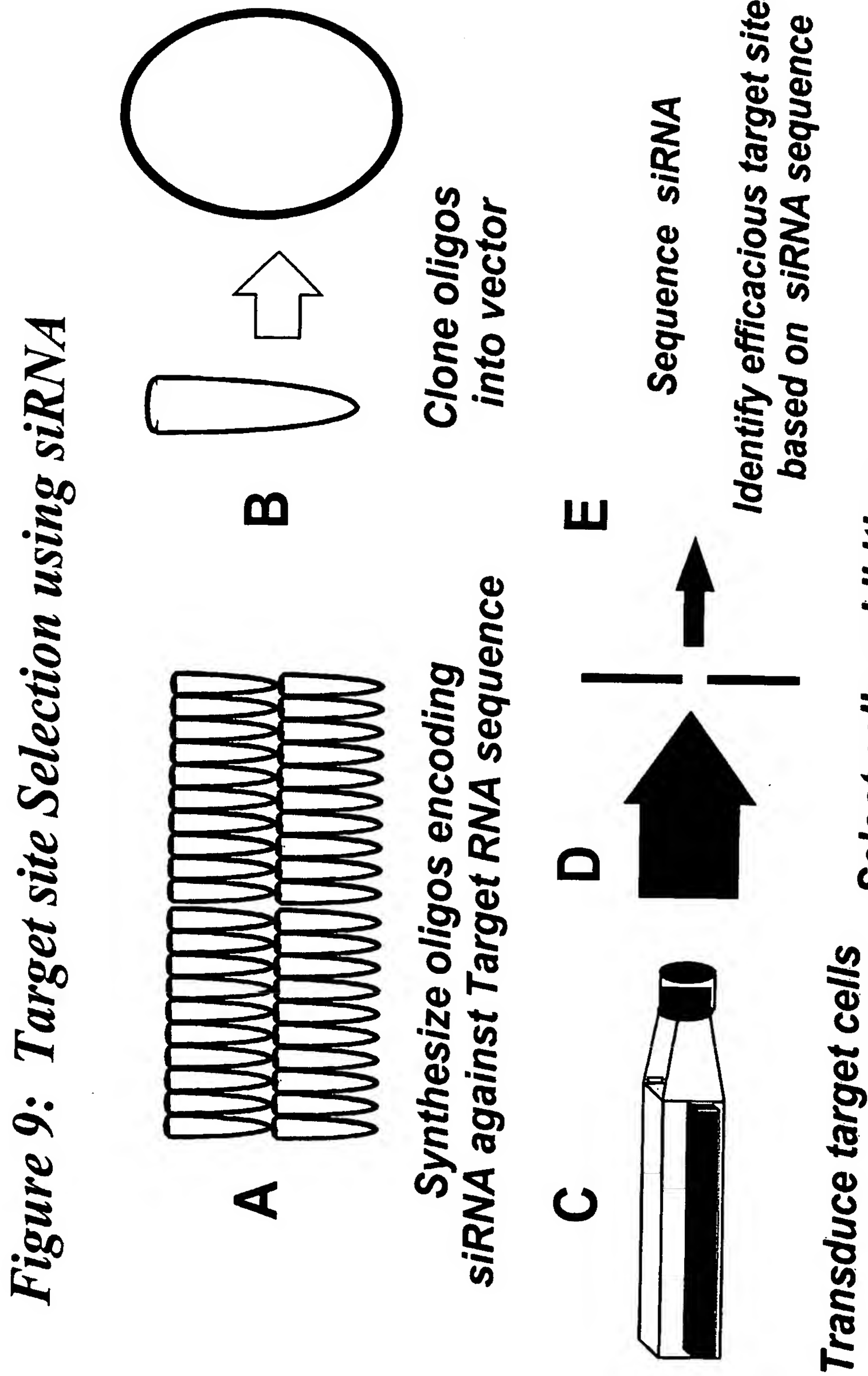
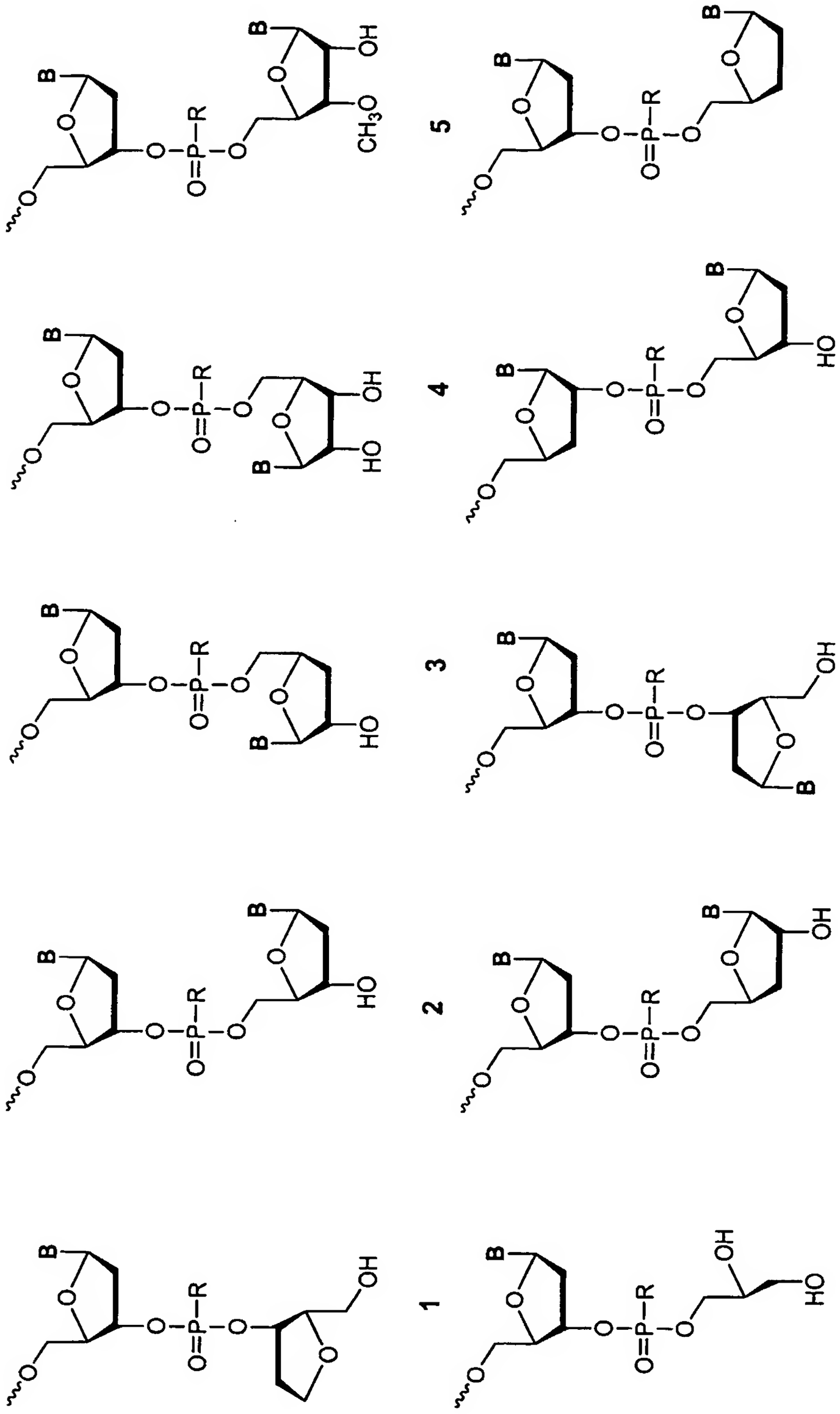


Figure 9: Target site Selection using siRNA

Figure 10



R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 11: Modification Strategy

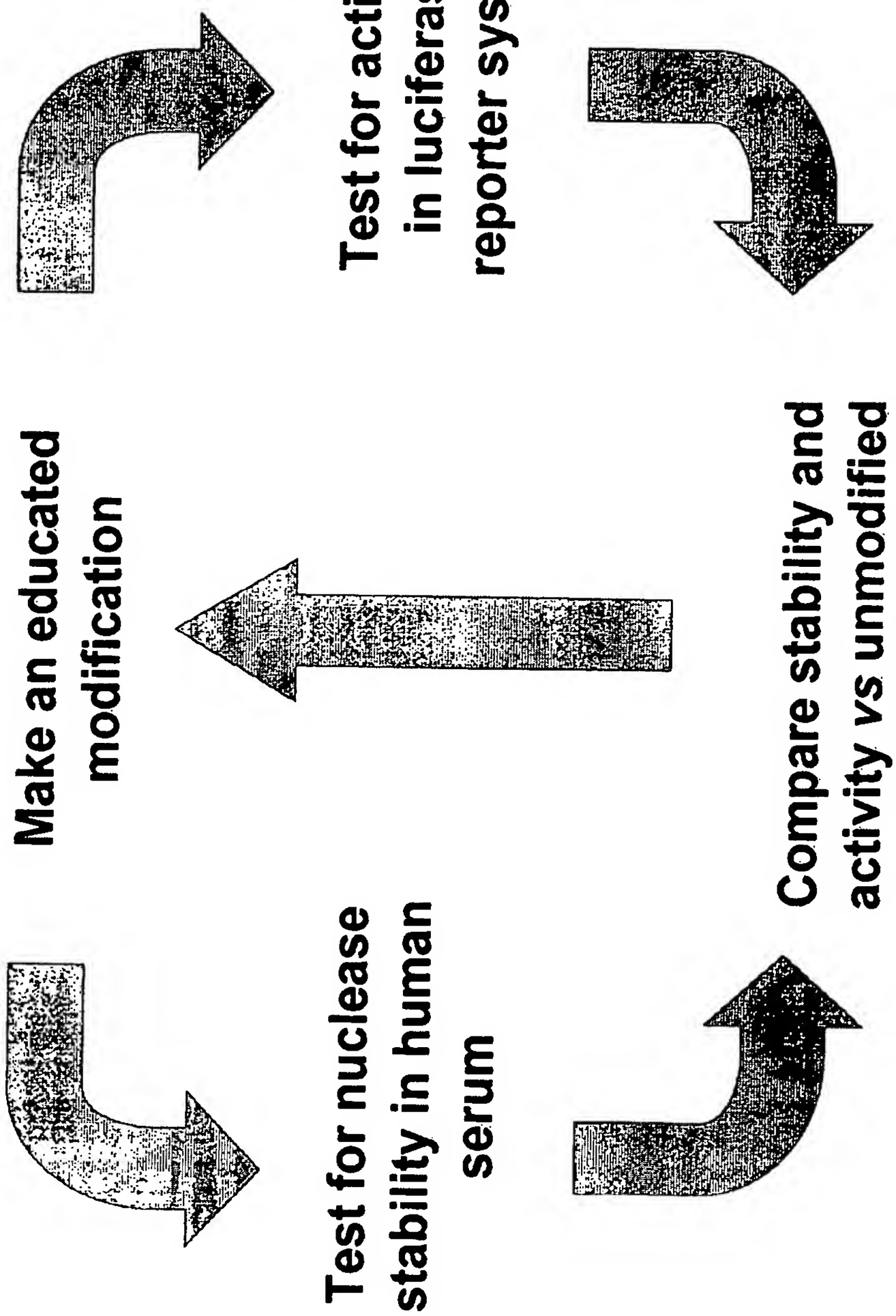
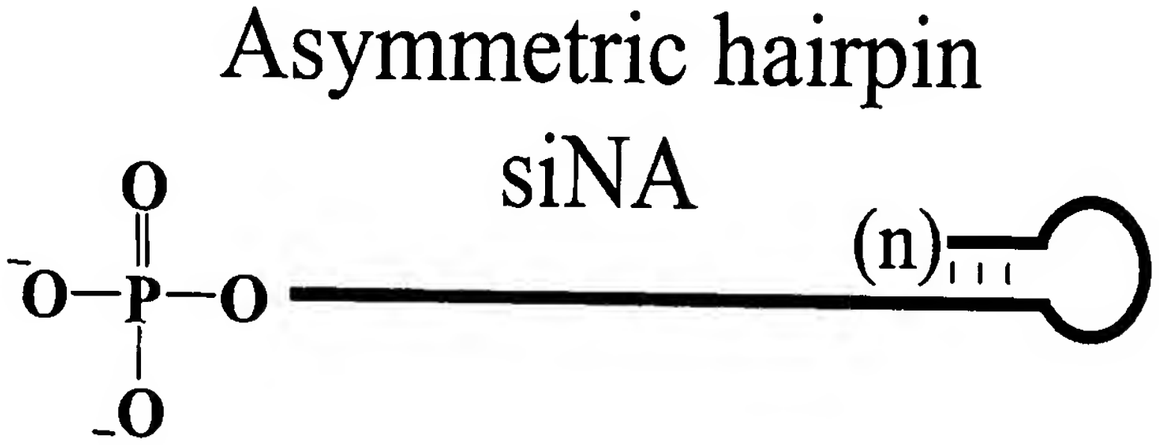
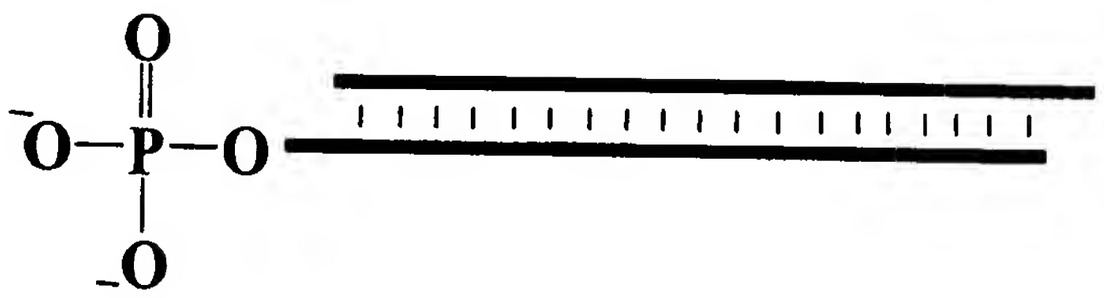


Figure 12: Phosphorylated siNA constructs

Phosphates can be modified as described herein





Asymmetric duplex siNA

(n) = number of base pairs (e.g. 3-18 bp)

Figure 13: 5'-phosphate modifications

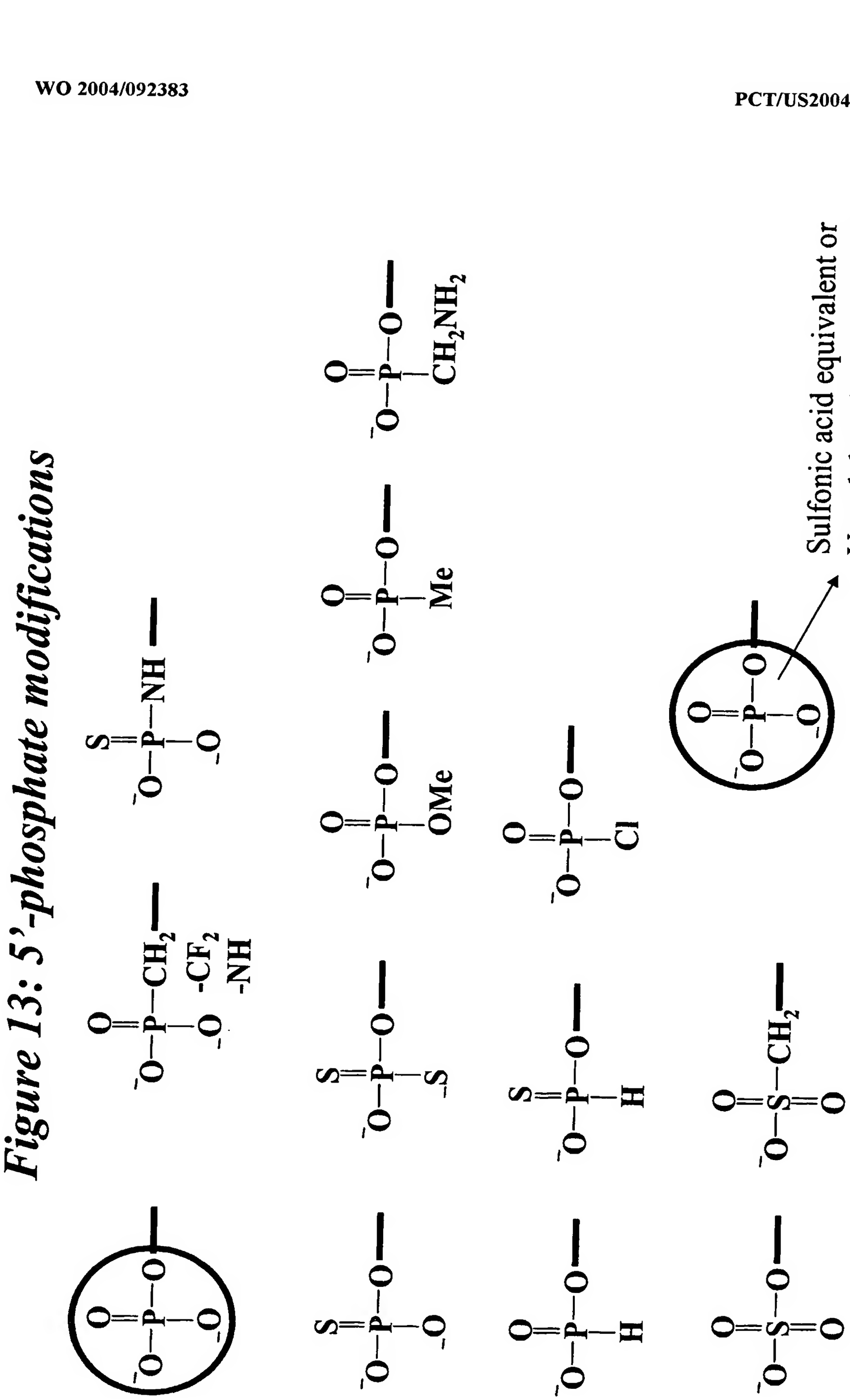
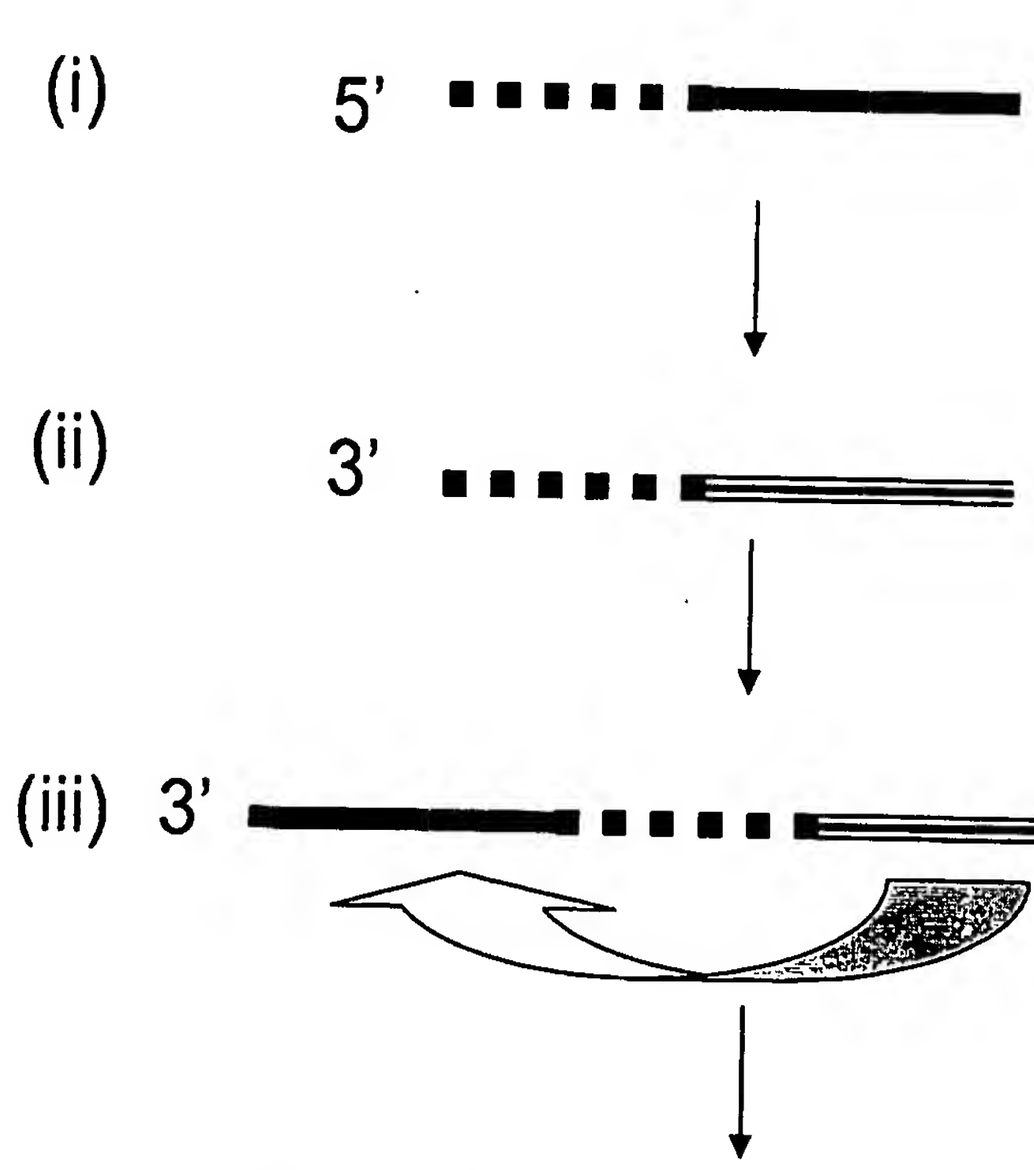


Figure 14A: Duplex forming oligonucleotide constructs that utilize palindrome or repeat sequences



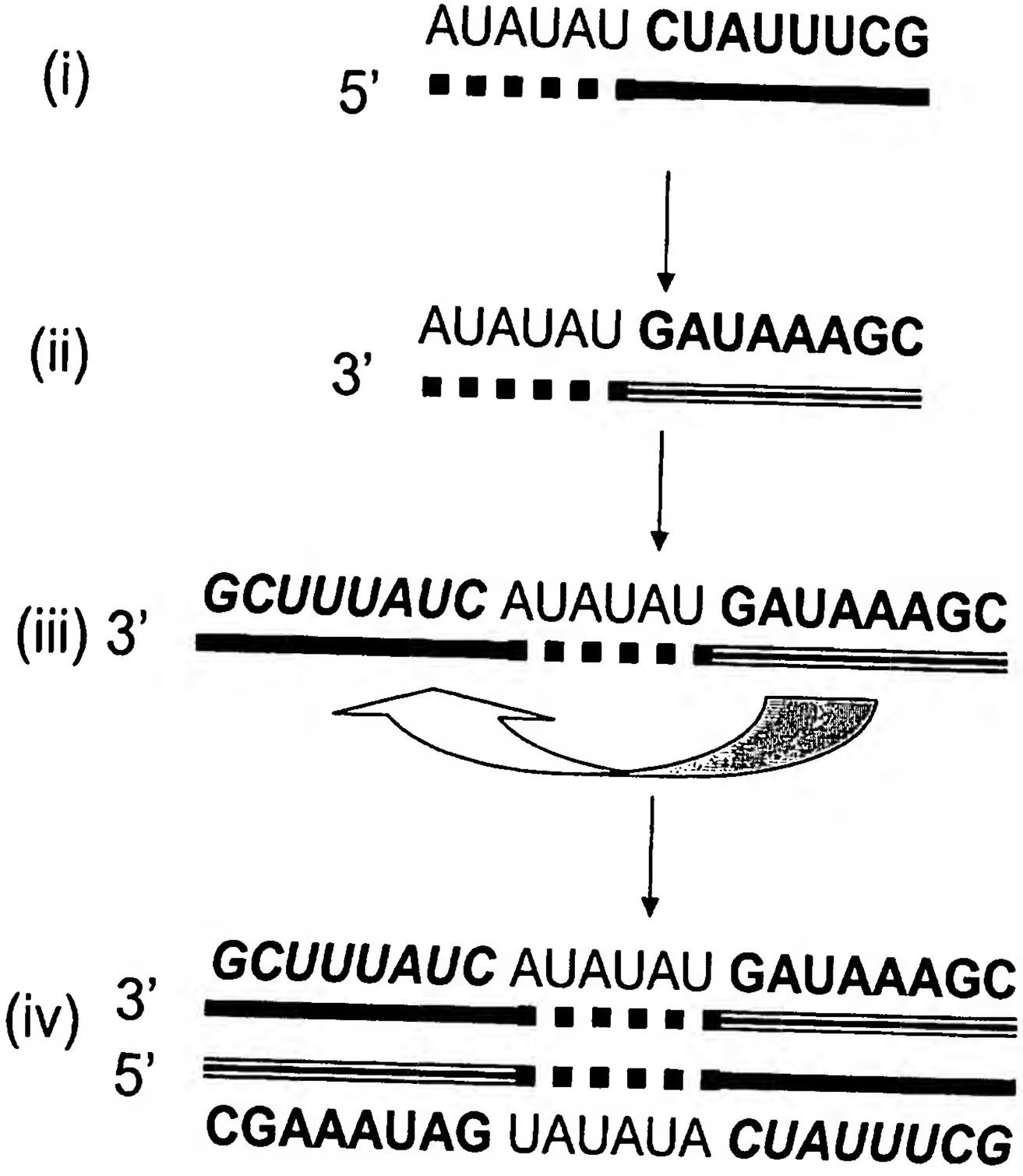
Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length) containing palindrome/repeat sequence at 5'-end (dashed portion)

Design Complementary Sequence to the Target Nucleic Acid sequence of (i) above

Append inverse sequence of the Non-palindromic Complementary Sequence of (ii) to 3'-end of complementary sequence

Self assembly of self complementary strands to form duplex construct

Figure 14B: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence



Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length) containing palindrome/repeat sequence at 5'-end (dashed portion)

Design Complementary Sequence to the Target Nucleic Acid sequence of (i) above

Append inverse sequence of the Non-palindromic Complementary Sequence of (ii) to 3'-end of complementary sequence

Self assembly of self complementary strands to form duplex construct (blunt ends)

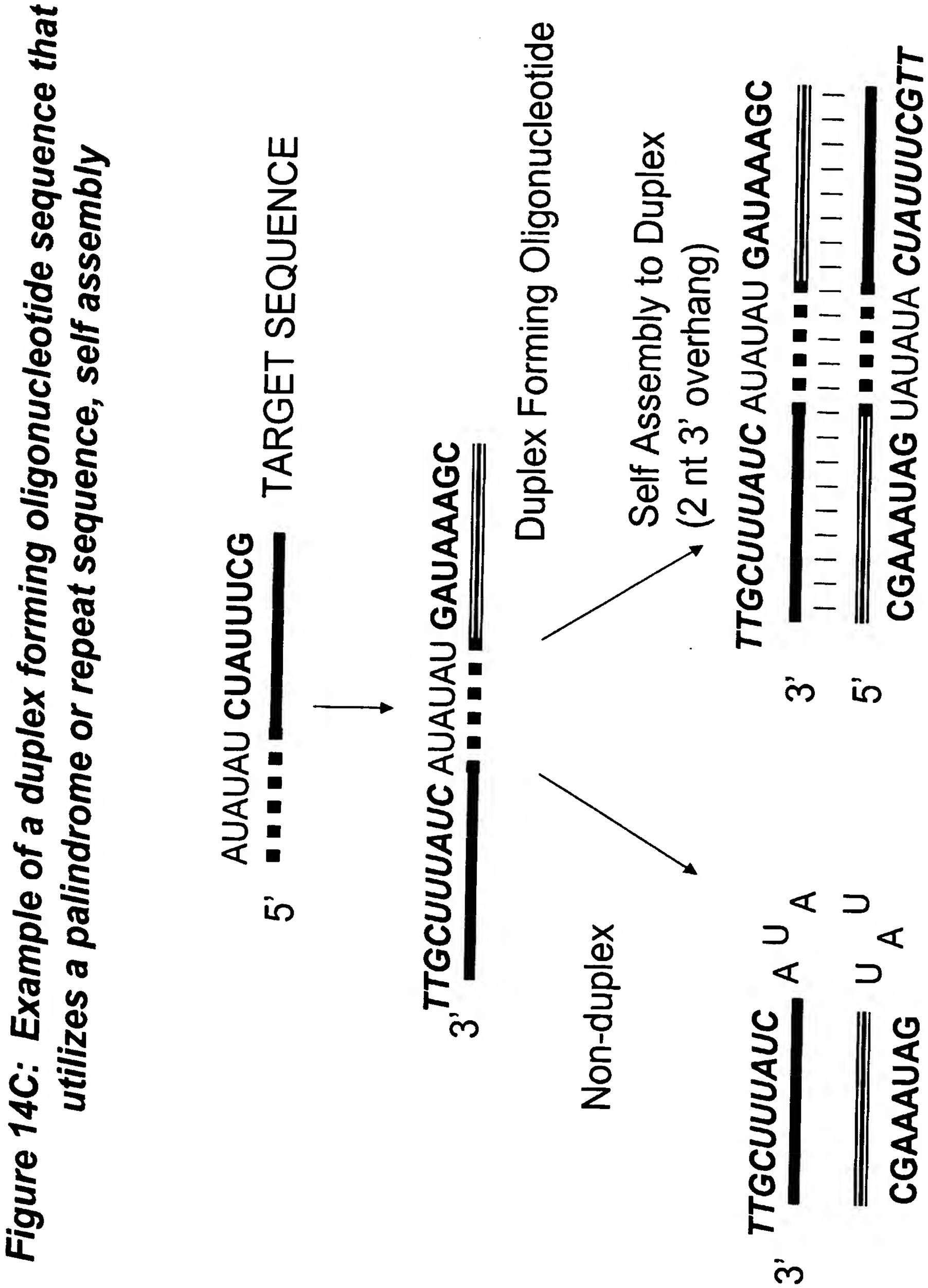
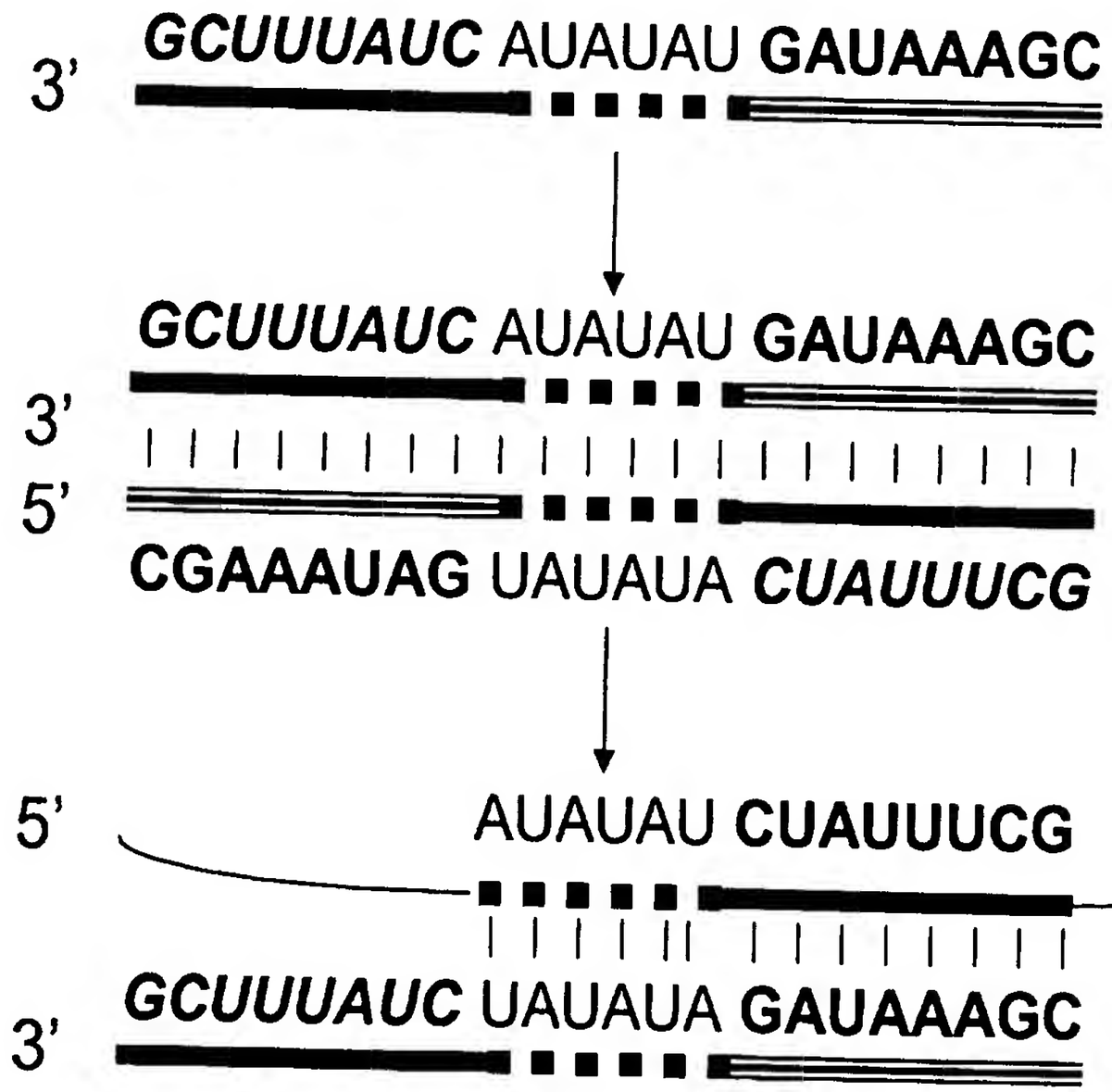


Figure 14C: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly

Figure 14D: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly and inhibition of Target Sequence Expression



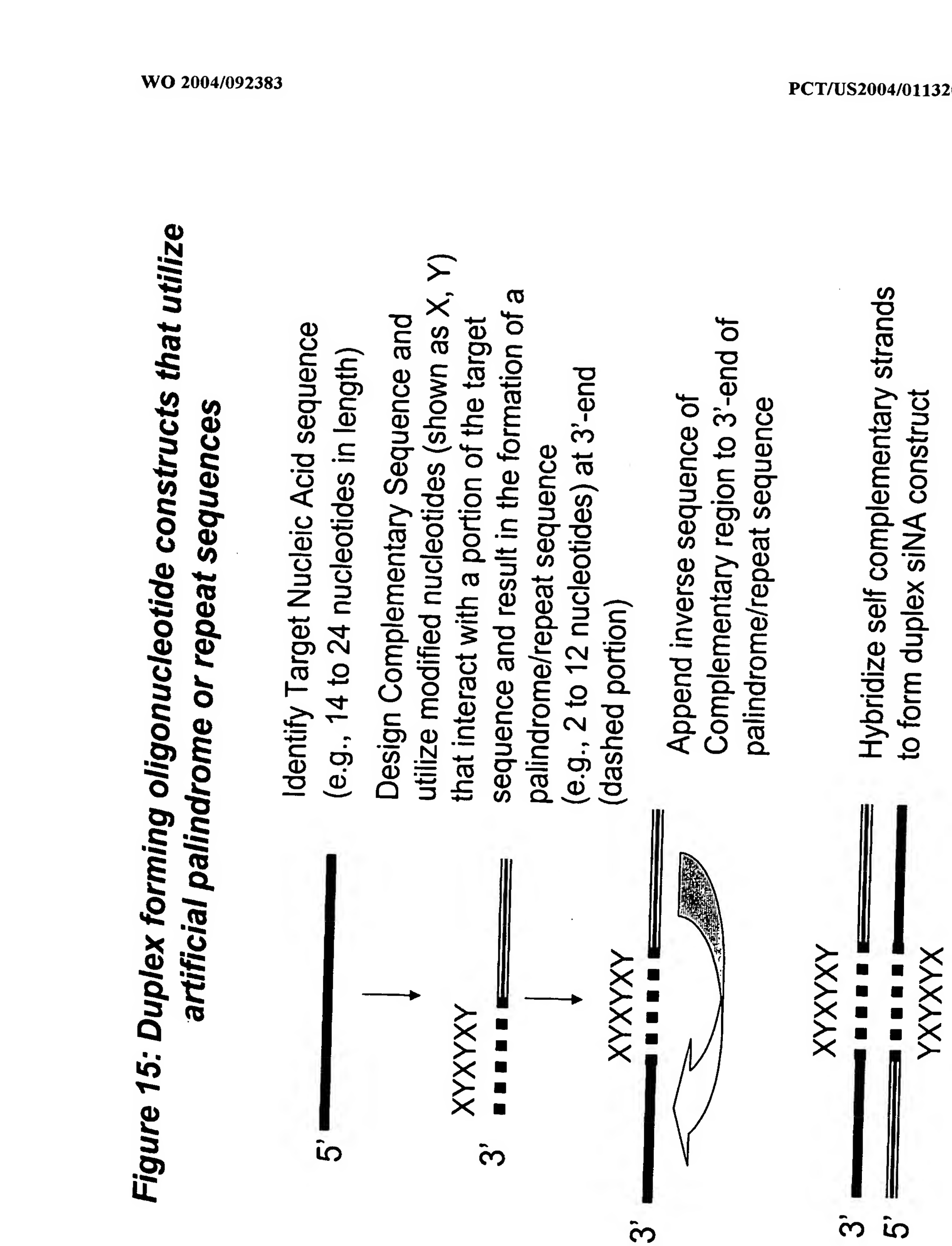
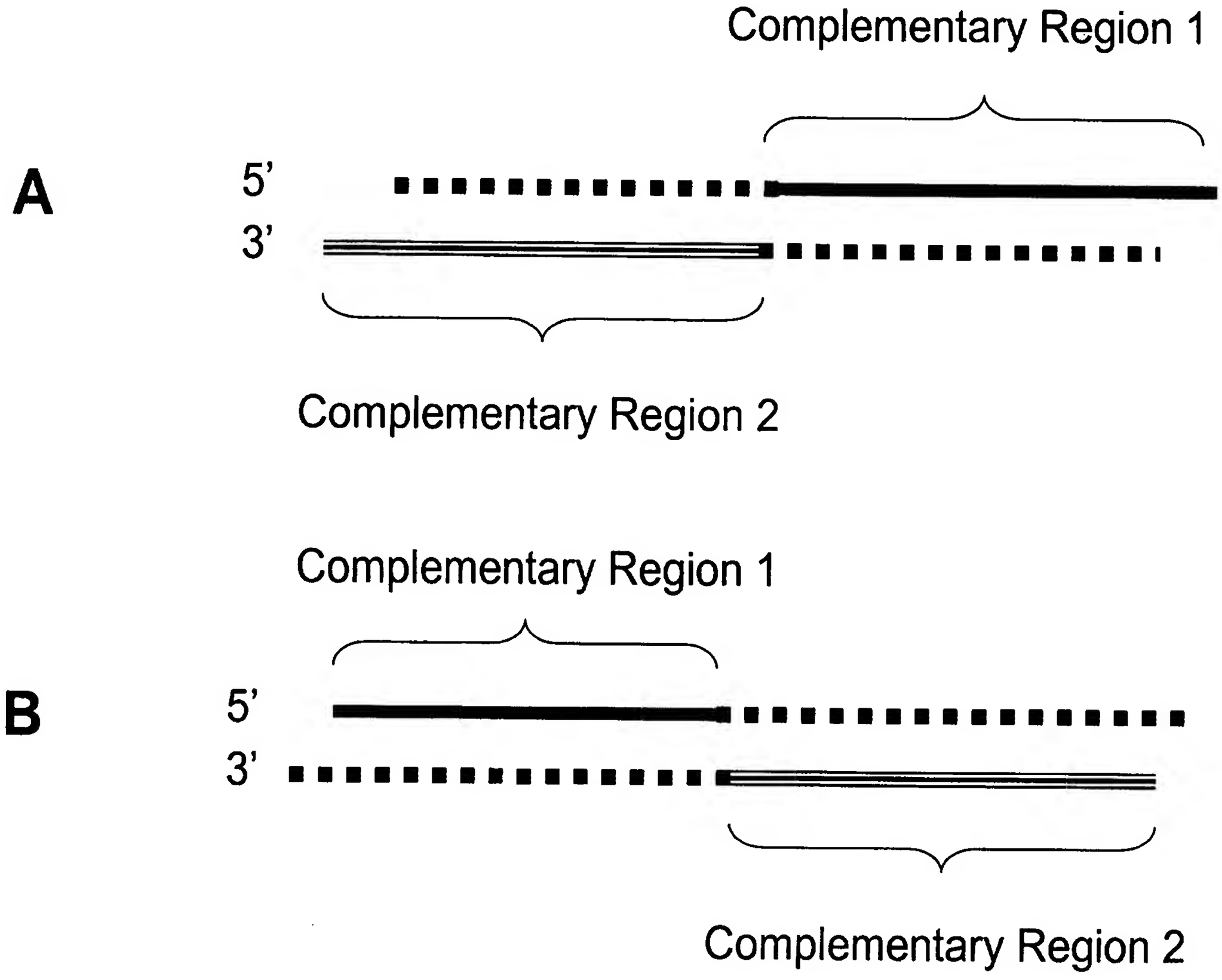


Figure 15: Duplex forming oligonucleotide constructs that utilize artificial palindrome or repeat sequences

Figure 16: Examples of double stranded multifunctional siNA constructs with distinct complementary regions



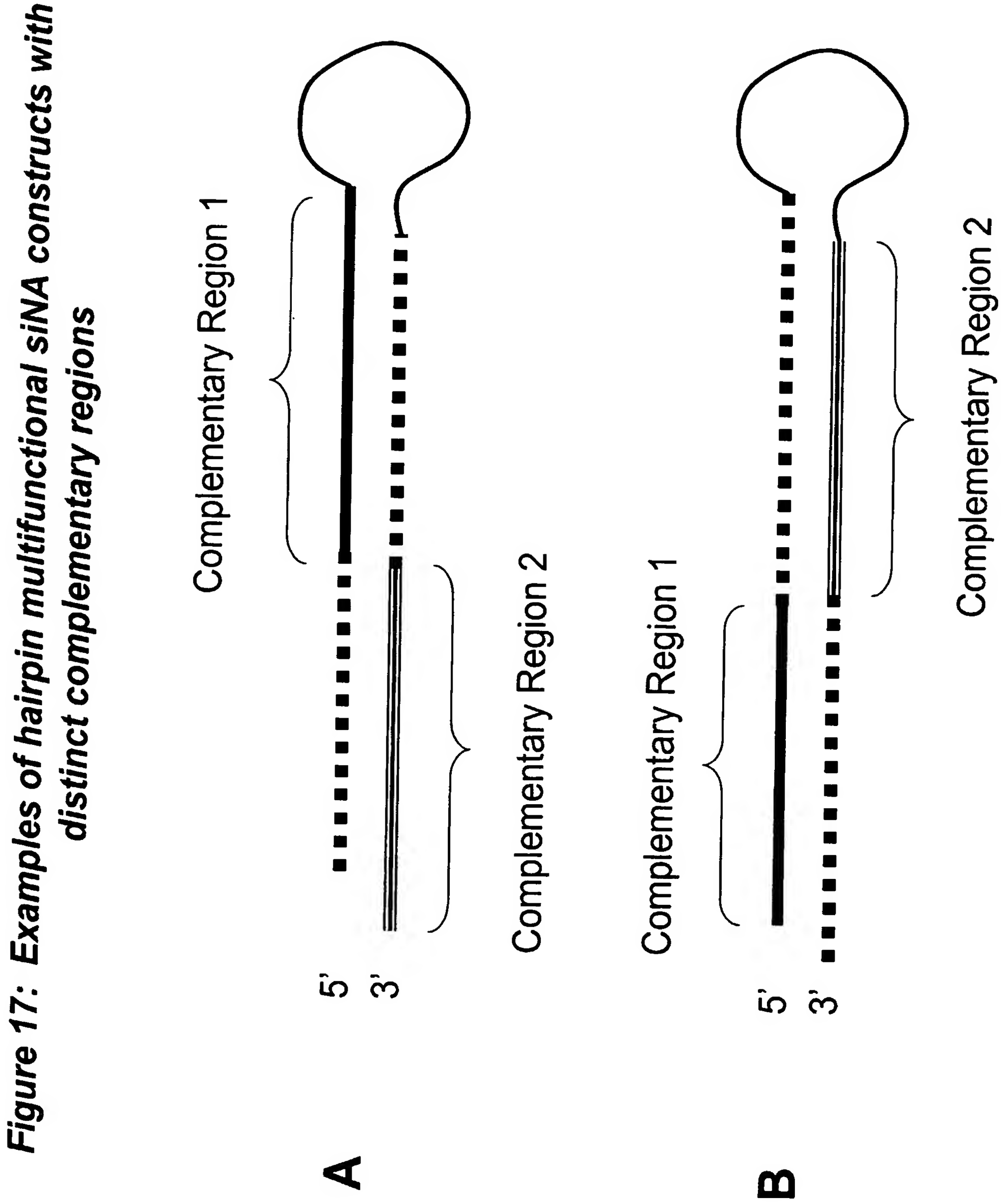


Figure 17: Examples of hairpin multifunctional siNA constructs with distinct complementary regions

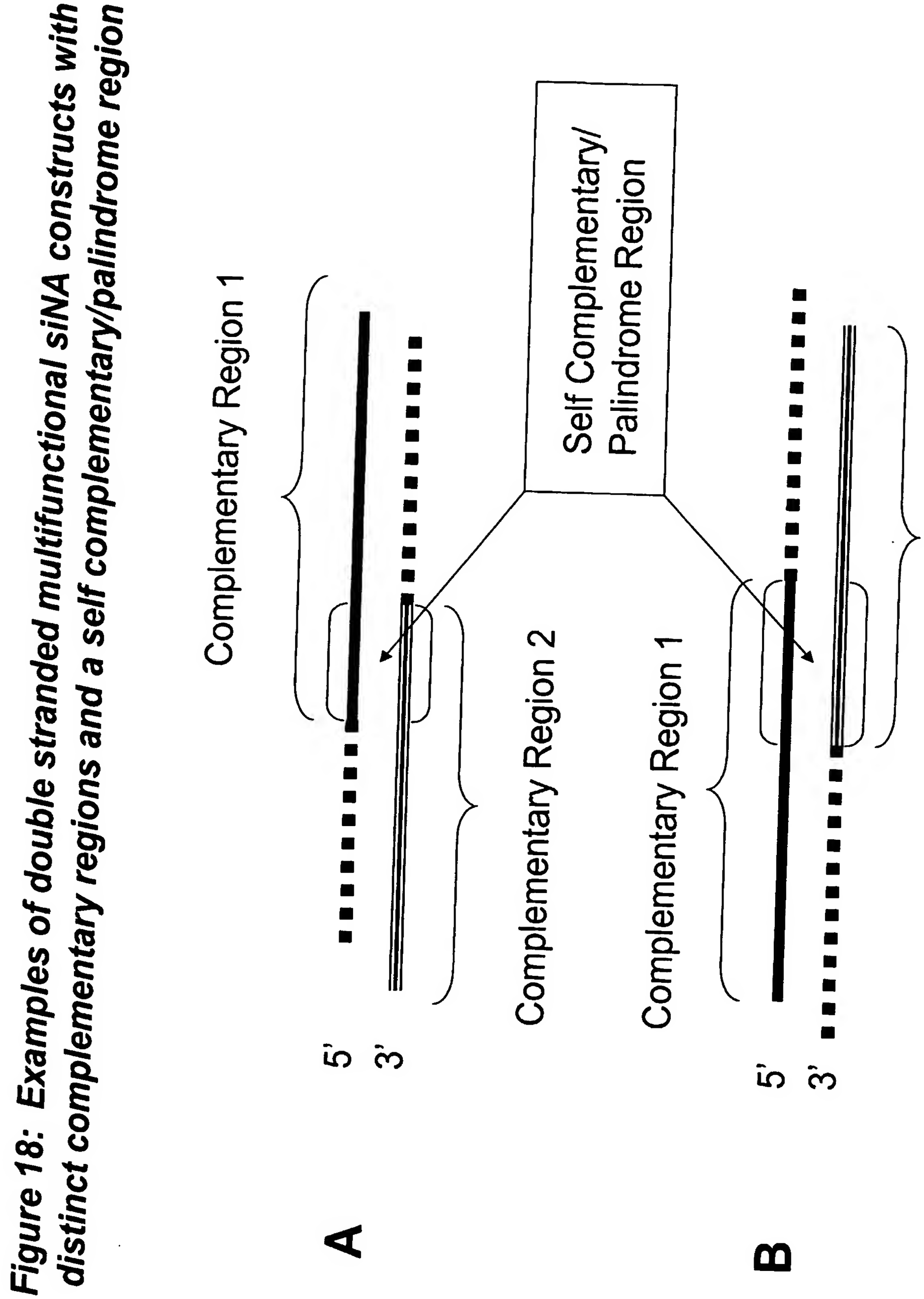
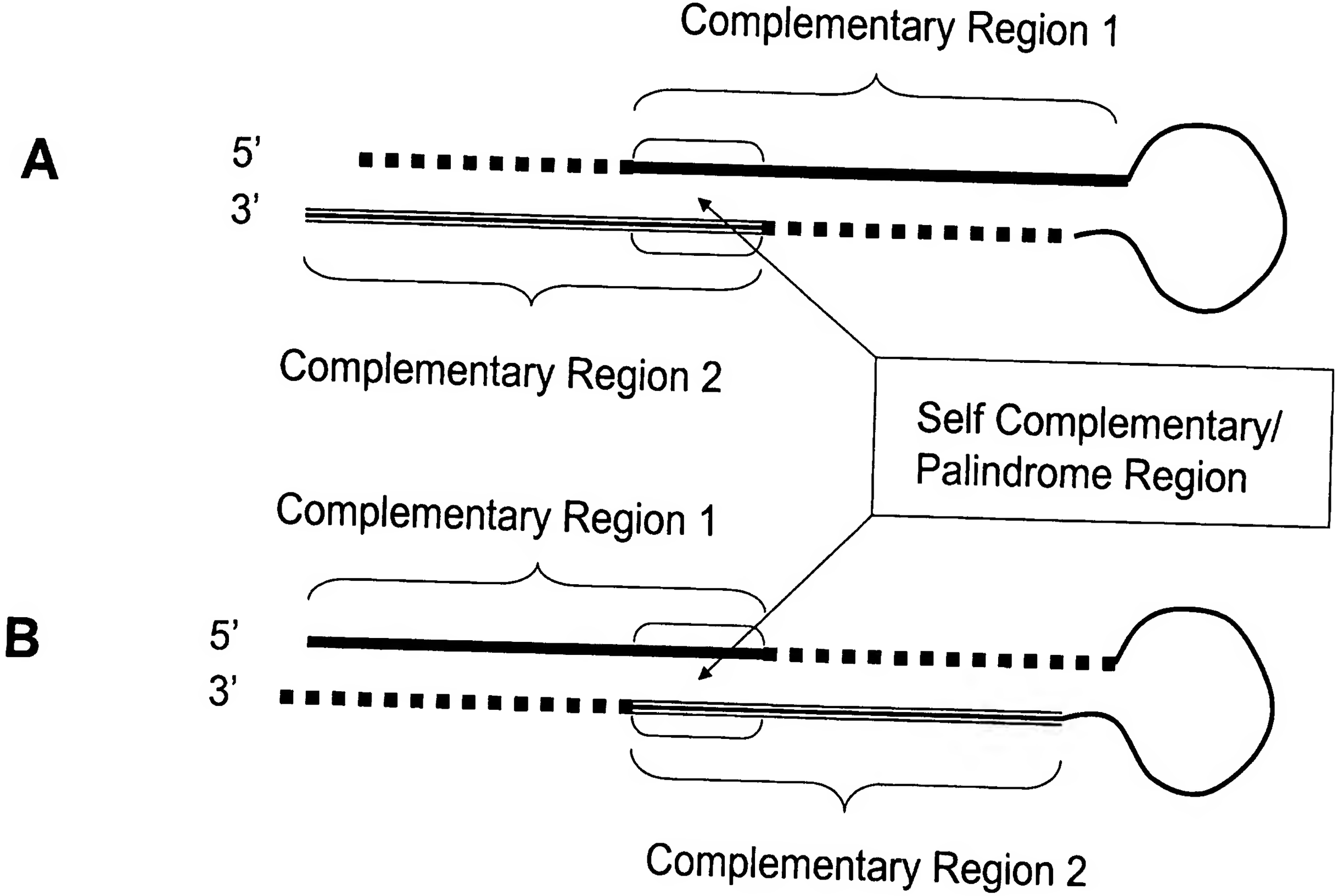


Figure 18: Examples of double stranded multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

Figure 19: Examples of hairpin multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region



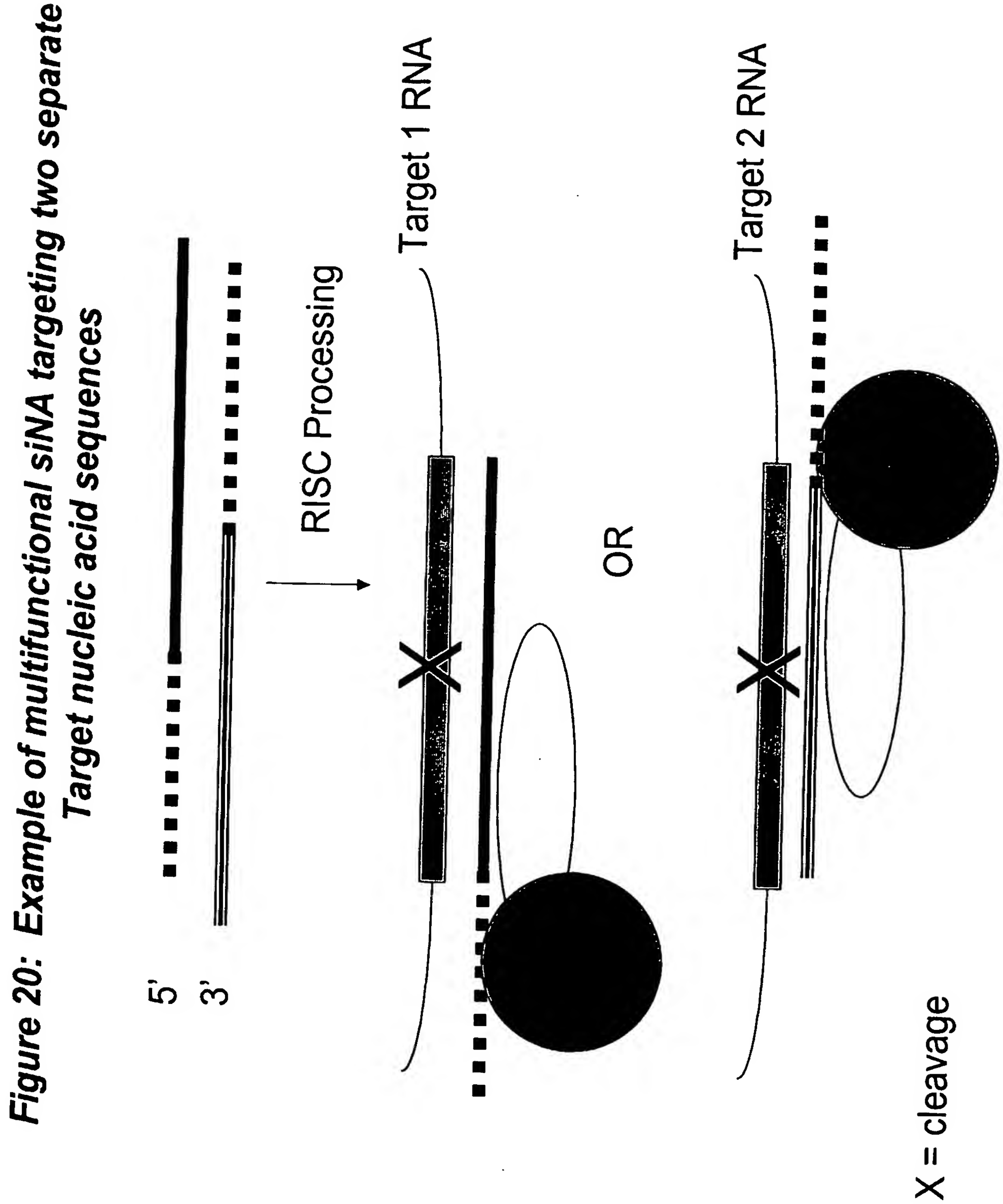


Figure 20: Example of multifunctional siNA targeting two separate Target nucleic acid sequences

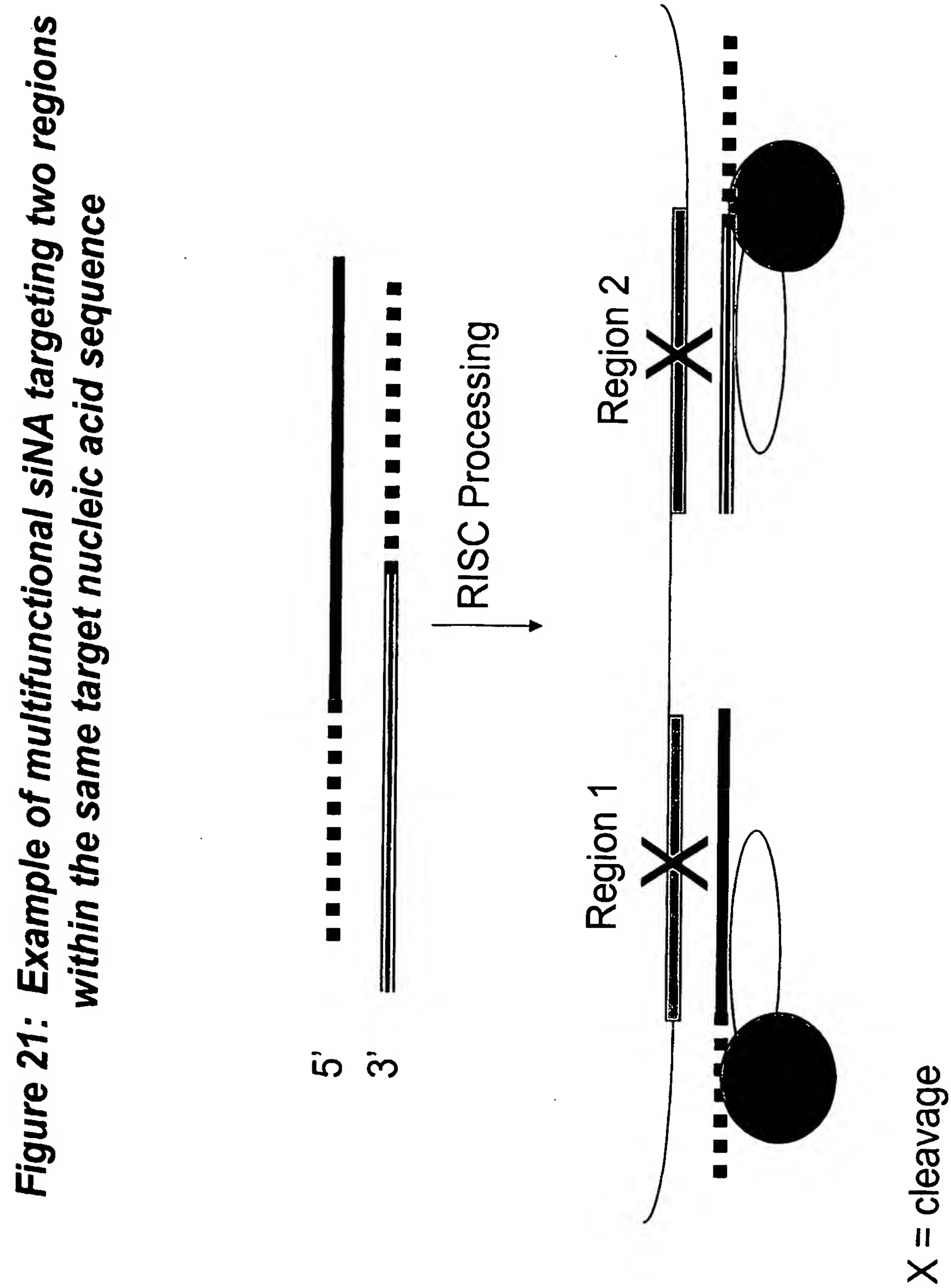


Figure 21: Example of multifunctional siNA targeting two regions within the same target nucleic acid sequence